IN THE CLAIMS:

Please amend claims 1, 7, 16, 45, 48, 50, 51, 52, 53, 54, 57, 58, 59, and 63 and add claims 67-78 as follows. Please cancel claims 60 and 64 without prejudice or disclaimer. This listing of claims replaces all prior versions, and listings of claims, in the application.

LISTING OF CLAIMS:

1. (Currently Amended) A method of producing and identifying a mammalian protease mutein that inactivates an activity of ~~with increased cleavage activity and/or altered substrate specificity for~~ a target protein involved with a disease or pathology in a mammal, wherein:

the target protein is selected from among a cell surface molecule that transmits an extracellular signal for cell proliferation, a cytokine, a cytokine receptor and a signaling protein that regulates apoptosis whose inactivation can ameliorate a disease or pathology;

cleavage of a substrate sequence in [[said]] the target protein inactivates an activity of the target protein ~~serves as a treatment for said pathology~~; and

the method comprises the steps of:

(a) producing a library of protease muteins of a protease scaffold and/or catalytically active portions thereof, wherein:

each different mutein protease in the library is a member of the library;

each member of the library has N mutations relative to a wild-type mammalian protease scaffold or a catalytically active portion thereof; and

N is a positive integer;

(b) contacting members of the library with the target protein or with a polypeptide comprising a substrate sequence that is present in the target protein;

~~(b)~~ (c) measuring a cleavage activity and/or substrate specificity of at least two members of the library for the target protein or substrate sequence; and

~~(c)~~ (d) based on the measured activity and/or specificity, identifying members of the library that have ~~at least one mutein protease having~~ an increased cleavage activity and/or altered substrate specificity for cleaving ~~said~~ the substrate sequence ~~in~~ or the target protein, relative to the wild-type mammalian protease scaffold, thereby identifying a protease mutein or a catalytically active portion thereof that inactivates an activity of a target protein that is involved with the disease or pathology, whereby the identified protease is a candidate therapeutic for treatment of the disease or pathology.

2. (Previously presented) The method of claim 1, wherein the wild-type mammalian protease scaffold is a serine or cysteine protease.

3. (Previously presented) The method of claim 1, wherein N is an integer between 1 and 20.

4. (Previously presented) The method of claim 3, wherein N is an integer from 1-5.

5. (Previously presented) The method of claim 3, wherein N is an integer from 5-10.

6. (Previously presented) The method of claim 3, wherein N is an integer from 10-20.

7. (Currently Amended) The method of claim 1, wherein the wild-type mammalian protease is selected from among trypsin, chymotrypsin, subtilisin, MTSP-1, granzyme A, granzyme B, and granzyme M, elastase, chymase, papain, neutrophil elastase, complement factor serine proteases, ADAMTS13, neural endopeptidase/neprilysin, furin, [[and]] cruzain, and urokinase plasminogen activator (uPA).

8. (Cancelled)

9. (Previously presented) The method of claim 1, wherein the pathology is selected from among rheumatoid arthritis, sepsis, cancer, acquired immunodeficiency syndrome, respiratory tract infections, influenza, cardiovascular disease and asthma.

10. (Canceled)

11. (Original) The method of claim 1, wherein the target protein is involved in apoptosis.

12. (Previously presented) The method of claim 11, wherein the target protein is caspase-3, VEGF or VEGF-R.

13. (Previously presented) The method of claim 1, wherein the specificity of the identified protease mutein for cleaving the substrate sequence is increased by at least 10-fold compared to the specificity of the wild-type mammalian protease scaffold for cleaving the substrate sequence.

14. (Previously presented) The method of claim 1, wherein the specificity of the identified protease mutein for cleaving the substrate sequence is increased by at least 100-fold compared to the specificity of the wild-type mammalian protease scaffold for cleaving the substrate sequence.

15. (Previously presented) The method of claim 1, wherein the specificity of the identified protease mutein for cleaving the substrate sequence is increased by at least 1000-fold compared to the specificity of the wild-type mammalian protease scaffold for cleaving the substrate sequence.

16. (Currently amended) The method of claim 1, further comprising the steps of:

~~(d)~~ (e) identifying the mutation(s) contained in a first mutein protease and a second mutein protease identified in step c) as having increased cleavage activity and/or altered specificity;

~~(e)~~ (f) producing a third mutein protease containing the mutations of the first mutein protease and the mutations of the second mutein protease; and

~~(f)~~ (g) measuring the cleavage activity and/or substrate specificity of the third mutein protease to determine ~~whether~~ its cleavage activity and/or specificity for the substrate sequence compared to the first mutein protease or second mutein protease.

17-44. (Canceled)

45. (Currently amended) The method of claim 1, further comprising:

repeating steps a)-~~e)~~ d) iteratively to produce a further library of protease muteins from the identified muteins each with increased cleavage activity and/or altered substrate specificity; and

identifying among the further library, a mutein protease having altered specificity and/or increased cleavage activity relative to the wild-type mammalian protease.

46. (Cancelled)

47. (Cancelled)

48. (Currently amended) The method of claim 1, wherein the substrate sequence is a sequence in a target protein that is a human protein.

49. (Canceled)

50. (Currently amended) The method of claim 1, further comprising the steps of:

providing at least one mutein protease identified in step (d) ~~(e)~~; and

testing the mutein protease in a cell-based assay against a target protein comprising the substrate sequence for inactivation of an activity of the target protein.

51. (Currently amended) The method of claim 50, wherein the member of the library identified in step ~~(e)~~ (d) has the highest measured cleavage activity for the substrate sequence among the identified members of the library.

52. (Currently Amended) The method of claim 1, further comprising the steps of providing at least one mutein protease identified in step (d) ~~(c)~~, and testing the mutein protease in an *in vivo* assay for inactivation of an activity of the target protein.

53. (Currently amended) A method of producing and identifying a mammalian protease mutein that inactivates an activity of ~~with increased cleavage activity and/or altered substrate specificity for~~ a target protein involved with a disease or pathology in a mammal, wherein:

inactivation of the target protein can ameliorate a disease or pathology;

cleavage of a substrate sequence in [[said]] the target protein inactivates an activity of the target protein ~~serves as a treatment for said pathology~~;

the mammalian protease is selected from among a granzyme A, granzyme B, granzyme M, cathepsin, trypsin, chymotrypsin, subtilisin, MTSP-1, elastase, chymase, tryptase, collagenase, papain, neutrophil elastase, complement factor serine proteases, ADAMTS13, neural endopeptidase/neprilysin, furin, [[and]] cruzain, and urokinase plasminogen activator (uPA); and

the method comprises the steps of:

(a) producing a library of protease muteins of a protease scaffold and/or catalytically active portion thereof, wherein:

each different protease mutein in the library being a member of the library;[[,]]

each member of the library has ~~having~~ N mutations relative to a wild-type mammalian protease scaffold or a catalytically active portion thereof; [[,]] and

~~wherein~~ N is a positive integer;

(b) contacting members of the library with the target protein or with a polypeptide comprising a substrate sequence that is present in the target protein;

~~(b)~~ (c) measuring a cleavage activity and/or substrate specificity of at least two members of the library for the targer protein or substrate sequence;

~~(c)~~ (d) based on the measured activity and/or specificity, identifying those members of the library that have an ~~at least one protease mutein having~~ increased cleavage activity and/or altered substrate specificity for cleaving ~~said~~ the substrate sequence or the target protein relative to the wild-type mammalian protease scaffold, thereby identifying a protease mutein or a catalytically active porition thereof that inactivates an activity of a target protein that is involved with or causes the disease or pathology, whereby the identified protease is a candidate therapeutic for treatment of the disease or pathology;

~~(d)~~ (e) identifying the mutation(s) contained in a first mutein protease and a second mutein protease identified in step (d) ~~e)~~ as having increased cleavage activity and/or altered specificity for cleaving ~~said~~ the target protein or substrate sequence;

~~(e)~~ (f) generating a third mutein protease containing the mutations of the first mutein protease and the mutations of the second mutein; and

~~(f)~~ (g) measuring the cleavage activity and/or substrate specificity of the third mutein protease to determine whether the third mutein ~~produces a~~ protease ~~that~~ has increased cleavage activity and/or altered specificity toward the target protein or substrate sequence ~~and/or altered specificity for cleaving said substrate sequence~~ compared to the first mutein protease or second mutein protease.

54. (Currently amended) The method of claim 53, further comprising the steps of:

(g) providing at least one protease mutein identified in step (d) ~~(c)~~; and

(h) testing the protease mutein in a cell-based assay against a target protein comprising the substrate sequence for inactivation of an activity of the target protein.

55. (Cancelled)

56. (Previously presented) The method of claim 54, wherein the cell-based assay is an *in vivo* assay.

57. (Currently amended) The method of claim 53, further comprising:

repeating steps a)-~~c)~~) d) iteratively to produce a further library of protease muteins from the identified muteins each with increased cleavage activity and/or altered substrate specificity; and

identifying among the further library, a mutein protease having altered specificity and/or increased cleavage activity for the substrate sequence relative to the wild-type mammalian protease.

58. (Currently amended) The method of claim 53, wherein the substrate sequence is a sequence in a target protein that is a human protein.

59. (Currently amended) A method of producing and identifying a human protease mutein that inactivates an activity of ~~with increased cleavage activity and/or altered substrate specificity for~~ a target protein involved with a disease or pathology in a human, wherein:

cleavage of a substrate sequence in ~~said~~ the target protein inactivates an activity of the target protein ~~serves as a treatment for said pathology~~;

the target protein is selected from among a cell surface molecule that transmits an extracellular signal for cell proliferation, a cytokine, a cytokine receptor and a signaling protein that regulates apoptosis whose inactivation can ameliorate a disease or pathology; and

the method comprises the steps of:

(a) producing a library of protease muteins of a protease scaffold and/or catalytically active portions thereof, wherein:[[,]]

each different protease mutein in the library ~~being~~ is a member of the library;[[,]]

each member having N mutations relative to a wild-type human protease scaffold or a catalytically active portion thereof, wherein[[:]] N is a positive integer; and

the human protease scaffold is selected from among a granzyme A, granzyme B, granzyme M, cathepsin, trypsin, chymotrypsin, subtilisin, MTSP-1, elastase, chymase, tryptase, collagenase, papain, neutrophil elastase, complement factor serine proteases, ADAMTS13, neural endopeptidase/neprilysin, furin, [[and]] cruzain, and urokinase plasminogen activator (uPA);

(b) contacting members of the library with the target protein or with a polypeptide comprising a substrate sequence that is present in the target protein;

~~(b)~~ (c) measuring a cleavage activity and/or substrate specificity of at least two members of the library for the substrate sequence or the target protein; and

~~(c)~~ (d) based on the measured activity and/or specificity, identifying those members of the library that have ~~at least one protease mutein having~~ an increased cleavage activity and/or altered substrate specificity for cleaving ~~said~~ the substrate sequence or the target protein[[,]] relative to the wild-type human protease scaffold, thereby identifying a protease mutein o a catalytically active portion thereof that inactivates an activity of a target protein that is involved with or causes the disease or pathology, whereby the identified protease is a candidate therapeutic for treatment of the disease or pathology.

60. (Cancelled)

61. (Previously presented) The method of claim 59, wherein the wild-type protease scaffold is Granzyme B or MTSP-1.

62. (Previously presented) The method of claim 59, wherein the target protein is selected from among caspase 3, tumor necrosis factor, tumor necrosis factor receptor, interleukin-1, interleukin-1 receptor, interleukin-2, interleukin-2 receptor, interleukin-4, interleukin-4 receptor, interleukin-5, interleukin-5 receptor, interleukin-12, interleukin-12

receptor, interleukin-13, interleukin-13 receptor, p-selectin, p-selectin glycoprotein ligand, Substance P, Bradykinin, PSGL, factor IX, immunoglobulin E, immunoglobulin E receptor, CCR5, CXCR4, glycoprotein 120, glycoprotein 41, hemagglutinin, respiratory syncytium virus fusion protein, B7, CD28, CD2, CD3, CD4, CD40, vascular endothelial growth factor, VEGF receptor, fibroblast growth factor, endothelial growth factor, EGF receptor, TGF receptor, transforming growth factor, Her2, CCR1, CXCR3, CCR2, Src, Akt, Bcl-2, BCR-Abl, glucagon synthase kinase-3, cyclin dependent kinase-2 (cdk-2) and cyclin dependent kinase-4 (cdk-4).

63. (Currently amended) A method of producing and identifying a human protease mutein that inactivates an activity of ~~with increased cleavage activity and/or altered substrate specificity for~~ a target protein involved with a disease or pathology in a mammal, wherein:

cleavage of a substrate sequence in the ~~said~~ target protein inactivates an activity of the target protein ~~serves as a treatment for said pathology~~;

the target protein is selected from among a caspase 3, tumor necrosis factor, tumor necrosis factor receptor, interleukin-1, interleukin-1 receptor, interleukin-2, interleukin-2 receptor, interleukin-4, interleukin-4 receptor, interleukin-5, interleukin-5 receptor, interleukin-12, interleukin-12 receptor, interleukin-13, interleukin-13 receptor, p-selectin, p-selectin glycoprotein ligand, Substance P, Bradykinin, PSGL, factor IX, immunoglobulin E, immunoglobulin E receptor, CCR5, CXCR4, glycoprotein 120, glycoprotein 41, hemagglutinin, respiratory syncytium virus fusion protein, B7, CD28, CD2, CD3, CD4, CD40, vascular endothelial growth factor, VEGF receptor, fibroblast growth factor, endothelial growth factor, EGF receptor, TGF receptor, transforming growth factor, Her2, CCR1, CXCR3, CCR2, Src, Akt, Bcl-2, BCR-Abl, glucagon synthase kinase-3, cyclin dependent kinase-2 (cdk-2), and cyclin dependent kinase-4 (cdk-4) whose inactivation can ameliorate a disease or pathology; and

the method comprises the steps of:

(a) producing a library of human protease muteins of a protease scaffold and/or catalytically active portions thereof, wherein:

_____ each different protease mutein in the library is ~~being~~ a member of the library[[,]];

_____ each member having N mutations relative to a wild-type human protease scaffold or a catalytically active portion thereof, wherein[[:]] N is a positive integer; and

the human protease scaffold is selected from among a granzyme A, granzyme B, granzyme M, cathepsin, MTSP-1, elastase, chymase, tryptase, chymotrypsin, collagenase, factor Xa, Protein C, plasma kallikrein, plasmin, trypsin, thrombin, complement factor serine proteases, papain, ADAMTS13, endopeptidase, furin, cruzain and urokinase plasminogen activator (uPA); and

(b) contacting members of the library with the target protein or with a polypeptide comprising a substrate sequence that is present in the target protein;

~~(b)~~ (c) measuring a cleavage activity and/or substrate specificity of at least two members of the library for the substrate sequence or the target protein; and

~~(c)~~ (d) based on the measured activity and/or specificity, identifying ~~at least one protease mutein having~~ those members of the library that have an increased cleavage activity and/or altered substrate specificity for cleaving ~~said~~ the substrate sequence or the target protein[[,]] relative to the wild-type human protease scaffold, thereby identifying a protease mutein or a catalytically active portion thereof that inactivates an activity of a target protein that is involved with the disease or pathology, whereby the identified protease is a candidate therapeutic for treatment of the disease or pathology.

64. (Cancelled)

65. (Previously presented) The method of claim 63, wherein the human protease scaffold is selected from among granzyme A, granzyme B, granzyme M and MTSP-1.

66. (Previously presented) The method of any one of claims 63-65, wherein the target protein is selected from among caspase 3, vascular endothelial growth factor and VEGF receptor.

67. (New) The method of claim 1, wherein the substrate sequence is pre-selected so that its cleavage in the target protein inactivates the target protein.

68. (New) The method of claim 1, wherein the substrate sequence is a tetrapeptide.

69. (New) The method of claim 1, wherein the target protein or substrate sequence is fluorgenically labeled.

70. (New) The method of claim 53, wherein the substrate sequence is pre-selected so that its cleavage in the target protein inactivates the target protein.

71. (New) The method of claim 53, wherein the substrate sequence is a tetrapeptide.

72. (New) The method of claim 53, wherein the target protein or substrate sequence is fluorgenically labeled.

73. (New) The method of claim 59, wherein the substrate sequence is pre-selected so that its cleavage in the target protein inactivates the target protein.

74. (New) The method of claim 59, wherein the substrate sequence is a tetrapeptide.

75. (New) The method of claim 59, wherein the target protein or substrate sequence is fluorgenically labeled.

76. (New) The method of claim 63, wherein the substrate sequence is pre-selected so that its cleavage in the target protein inactivates the target protein.

77. (New) The method of claim 63, wherein the substrate sequence is a tetrapeptide.

78. (New) The method of claim 63, wherein the target protein or substrate sequence is fluorgenically labeled.